Amendments to the Specification:

Please amend the paragraph beginning on page 9, line 14 as indicated.

--Prior to the present invention, unsuccessful attempts had been made at isolating insect Type III AFPs genes. Tang and Baust made use of an antiserum generated against an antifreeze protein active solution derived from *T. molitor*, designated AFP-3 (homogeneity of this peptide was not confirmed) to screen a cDNA *T. molitor* library and isolated a full length clone, the sequence of which was entered into Genbank ~~(NCBI Seq. ID: 785071)~~ (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071). This clone was prematurely (or even incorrectly) listed as an antifreeze protein since recombinant products did not display thermal hysteretic activity. Further support that the AFP-3 clone may not be an antifreeze protein comes from extensive studies by P. Davies and coworkers (Rothemund S. et al., [1997] Biochemistry 36:13791-13801]; [1999] Structure 7:1325-1332), molecular biology experts. In numerous attempts they have cloned the insert generated by Tang and expressed in bacteria the encoded peptide they designated as THP-12 (also known as AFP-3). The recombinant product in all attempts did not display any thermal hysteretic activity, and subsequent NMR spectroscopy studies suggest that the protein has a nonbundle helical structure consisting of six alpha helices arranged in a `baseball glove` shape (i.e. with no obvious ice binding motif seen). They have concluded that THP-12 (AFP-3) might be a member of small lipid carrier class of proteins, yet it[[']]s biological function is as yet undetermined.--

Please amend the sentence beginning on page 14, line 10 as indicated.

--A general process flow diagram for the present invention can be found in FIG. 1.0.--

Please amend the paragraph beginning on page 15, line 1 as indicated.

--The invention details further the relatedness of this Tm 12.86 AFP multigene family to other known genes, through Genbank searches, establishing that the proteins derived from the Tm 12.84 like clones and Tm 13.17 clone are most closely related (nucleic acid similarity, 43% and 57%, respectively) to B1/B2 accessory gland tubular proteins of adult male *T. molitor*. Also, they are somewhat similar in composition (42% and 37% for Tm 12.84 like and Tm 13.17, respectively) to a lipid carrying protein from Tenebrio designated AFP-3/THP 12 (Tang and Baust, [1995] Genbank NCBI ~~Seq ID: 785071~~ SEQ ID NO:785071; Rothemund et al., [1999]). Despite the latter protein's suggestive abbreviations, the current assessment of it is not that of an antifreeze protein (Rothemund et al., 1999). Finally, the Tm 12.86 AFP family shows no similarity (20%) to the recently isolated Type II AFPs from *T. molitor* and D. canadensis.--

Please amend the sentence beginning on page 16, line 37 as indicated.

--Other uses include, but are not limited to the cosmetic field, and cryosurgery. Additionally, all these effects can be mediated by adding purified THPs alone, or alternatively the THPs can be combined with various "enhancing ~~activitor~~ activator" or adjuvant compounds that are known to enhance THP activity.--

Please amend the sentence beginning on page 20, line 1 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 1.8 is the N-terminal analysis of Tm 12.86 ~~(SEQ. ID No. 1)~~ (SEQ ID NO:1) depicting leucine at the amino terminus.--

Please amend the sentence beginning on page 21, line 37 as indicated.

--Inserted cDNA can be excised by co-infection with helper phage from the ZAP express vector as a recombinant

~~Bluescript~~® BLUESCRIPT® SK (-) phagemid (plasmids with a phage origin, sold by Stratagene).--

Please amend the paragraph beginning on page 22, line 16 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 2.6 is a complete sequence of the FW1 clone encoding Tm 13.17 ~~(SEQ. ID NO. 2)~~ (SEQ ID NO:2) and its deduced amino acid (SEQ ID NO:3 and SEQ ID NO:4) ~~(SEQ. ID NOs 3 and 4)~~ of the protein of *T. molitor*. FIG. 2.6A is the full length nucleotide sequence and corresponding deduced amino acid (in single letter nomenclature); The translation start codon, ATG is boxed, and a putative signal peptide sequence are underlined; the stop codon. TGA is in asterisk; polyadenlation signal is in italic and bold, and poly (A) tail is in bold. The arrow indicates the putative cleavage site of the signal peptide. FIG. 2.6B is the signal peptide from deduced amino acid sequence of FW1 cDNA clone. The typical three regions of signal peptide are underlined. The cleavage site is indicated by arrow. FIG. 2.6C is the amino acid sequence and compositional analysis for the predicted mature Tm 13.17.--

Please amend the paragraph beginning on page 22, line 26 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 2.7 illustrates the alignment between the nucleotide cDNA sequences of B1 (Paesen, G. C. and G.M. Happ, [1995] Insect Biochem. Molec. Biol. 25: 401-408) and Tm 13.17 (SEQ ID NO:2) of *T. molitor*. Identical nucleotide sequence is boxed. The start of the mature protein is marked with an arrow, and the stop codons are shown by a star.--

Please amend the paragraph beginning on page 22, line 29 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 2.8 illustrates the sequence alignment between mature Tm 13.17 (SEQ ID NO:4) and AFP-3 (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071) of *T. molitor*. Vertical line indicates identical amino acids; two dots indicate highly conservative replacement, and one dot indicates less conservative replacement.--

Please amend the paragraph beginning on page 22, line 32 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 2.9 illustrates the alignment of putative signal peptide sequences of Tm 13.17 (SEQ ID NO:3), AFP-3 (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071) and B1 (Paesen, G. C. and G.M. Happ, [1995] Insect Biochem. Molec. Biol. 25: 401-408) protein of *T. molitor*. The identical amino acid residues and highly conservative replacement are boxed.--

Please amend the sentence beginning on page 22, line 35 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 2.10 illustrates the alignment of N-terminal amino acid sequences of Tm 13.17 (SEQ ID NO:4) and Tm 12.86 (SEQ ID NO:1). The identical amino acids are boxed, dots indicate conservative replacement amino acids.--

Please amend the paragraph beginning on page 23, line 7 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--Fig. 2.12 illustrates the alignment of three amino acid sequences for Tm 13.17 (SEQ ID NO:4), B1 (Paesen, G. C. and G.M. Happ, [1995] Insect Biochem. Molec. Biol. 25: 401-408), and AFP-3 (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071). 18 N-terminal amino acid residues of Tm 12.86 (SEQ ID NO:1) is also shown in the alignment. The identical amino acid residues are boxed. Note that the arrangement of the proteins from top to bottom (Tm 12.86 (SEQ ID NO:1), Tm 13.17

(SEQ ID NO:4), B1 (Paesen, G. C. and G.M. Happ, [1995] Insect Biochem. Molec. Biol. 25: 401-408), and AFP-3 (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071)) displays first the strong relatedness, and then the falling off identity between the peptides.--

Please amend the paragraph beginning on page 23, line 12 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 3.0 illustrates the cDNA nucleotide sequence ~~(SEQ ID NO 5)~~ (SEQ ID NO:5) and amino acid translation of clone 2-2 ~~(SEQ ID NO 7 AND 8)~~ (SEQ ID NO:7 and SEQ ID NO:8). The signal sequence is underlined, and the arrow denotes the predicted beginning of the mature protein. The start codon is boxed, and the stop codon is denoted by a star.--

Please amend the paragraph beginning on page 23, line 16 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 3.1 is the cDNA nucleotide sequence ~~(SEQ ID NO 6)~~ (SEQ ID NO:6) and amino acid translation of clone 2-3 ~~(SEQ ID NO 7 AND 8)~~ (SEQ ID NO:7 and SEQ ID NO:8). The signal sequence is underlined, and the arrow denotes the predicted beginning of the mature protein. The start codon is boxed, and the stop codon is denoted by a star.--

Please amend the paragraph beginning on page 23, line 20 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 3.2[[:]] illustrates comparative nucleotide sequence analysis (SEQ ID NO:5 and SEQ ID NO:6) between clones 2-2& 2-3. Areas of the sequences that are different are boxed.--

Please amend the paragraph beginning on page 23, line 22 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 3.3 illustrates predicted amino acid composition and related information for the peptide derived from clones 2-2/2-3 (SEQ ID NO:8).--

Please amend the paragraph beginning on page 24, line 33 as indicated.

--B. is identical blot to above, but hybridized with the 32P labeled 2-3 probe.--

Please amend the paragraph beginning on page 25, line 11 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.6 illustrates PCR primers used to amplify genomic DNA. FIG. 4.6A illustrates the Tm 13.17 cDNA nucleotide sequence, with the forward and reverse primer sequences boxed (SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4). FIG. 4.6B illustrates representative amino acid sequence alignments of 2-2 (SEQ ID NO:8), Tm 13.17 (SEQ ID NO:4), B2 (Paesen G. C., and G. M. Happ [1995] Insect Biochem. Molec. Biol. 25: 401-408), and AFP-3 (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071). The primer sequences, which only exactly match Tm 13.17, were taken from the boxed areas. FIG. 4.6C illustrates the percent composition and melting temperatures of the forward and reverse primers shown in FIG. 4.6A.--

Please amend the paragraph beginning on page 25, line 27 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.10A is the cDNA nucleotide sequence ~~(SEQ. ID NO. 9)~~ (SEQ ID NO:9) and translation of 3-4 ~~(SEQ ID NO. 10 (precursor) and SEQ. ID NO. 11 (mature protein)~~ (SEQ ID NO:10)

(precursor) and (SEQ ID NO:11) (mature protein). The signal sequence is underlined, and the arrow denotes the predicted beginning of the mature protein. The start codon is boxed, and the stop codon is denoted with a star. FIG. 4.10B is the amino acid composition and related information of the predicted mature 3-4 protein.--

Please amend the paragraph beginning on page 25, line 32 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.11A is the cDNA nucleotide sequence ~~(SEQ. ID NO. 12)~~ (SEQ ID NO:12) and translation of 3-9 ~~(SEQ ID NO. 13 (precursor) and SEQ. ID NO. 14 (mature protein)~~ (SEQ ID NO:13) (precursor) and (SEQ ID NO:14) (mature protein). The signal sequence is underlined, and the arrow denotes the predicted beginning of the mature protein. The start codon is boxed, and the stop codon is denoted with a star. FIG. 4.11B is the amino acid composition and related information of the predicted mature 3-9 protein.--

Please amend the paragraph beginning on page 25, line 37 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.12A is the cDNA nucleotide sequence ~~(SEQ. ID NO. 15)~~ (SEQ ID NO:15) and translation of 7-5 ~~(SEQ ID NO. 7 (precursor) and SEQ. ID NO. 8 (mature protein)~~ (SEQ ID NO:7) (precursor) and (SEQ ID NO:8) (mature protein). The signal sequence is underlined, and the arrow denotes the predicted beginning of the mature protein. The start codon is boxed, and the stop codon is denoted with a star. FIG. 4.12B is the amino acid composition and related information of the predicted mature 7-5 protein.--

Please amend the paragraph beginning on page 26, line 3 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.13 illustrates the alignment between the cDNA sequences 2-2, 2-3, 3-4, 3-9, and 7-5 (SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:9; SEQ ID NO:12; SEQ ID NO:15). Nucleotide residues which disagree are boxed. The start of the mature protein is denoted by an arrow, and the stop codon is marked with a star.--

Please amend the paragraph beginning on page 26, line 6 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.14 illustrates the alignment of the amino acid sequences of 2-2, 2-3, 3-4, 3-9, and 7-5 (SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:13; SEQ ID NO:14), predicted from the nucleotide sequence of the cDNAs. Amino acid residues that differ between sequences are boxed. The arrow denotes the start of the mature protein.--

Please amend the paragraph beginning on page 26, line 11 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.16 illustrates the alignment between the amino acid sequences of Tm 12.86, 2-2, 2-3, 3-4, 3-9, 7-5, Tm 13.17 (SEQ ID NO:1; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:3; SEQ ID NO:4), B1, B2 (Paesen G. C., and G. M. Happ [1995] Insect Biochem. Molec. Biol. 25: 401-408), and AFP-3 (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071). All are sequences obtained from *T. molitor*. All except Tm 12.86 are amino acid sequences predicted from cDNA nucleotide sequences. The start of the mature protein sequence is at the arrow. Conserved cysteine residues are denoted in yellow. Residues which agree in all nine sequences or ten including the N-terminus of Tm 12.86 are in blue. Residues which agree in at least seven proteins are in orange. An open circle denotes a single amino acid deletion in 2-2, 2-3, 3-4, 3-9 and 7-5.--

Please amend the paragraph beginning on page 26, line 18 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.17 illustrates the alignment of Tm 13.17 SEQ ID NO:4, 2-2 (SEQ ID NO:8) (representative of 2-2, 2-3, 3-4, 3-9, and 7-5), B1, B2 (Paesen G. C., and G. M. Happ [1995] Insect Biochem. Molec. Biol. 25: 401-408), and eight pheromone binding proteins from various insects (K. Raming, J. Kriegen, and H. Breer [1989] Federation Experimental Biology Letters 256:215-218; T.K. Gyoergyi, A.J. Robi-Shemkovitz and M.R. Lerner [1988] Proc Natn. Acad. Scil, USA 85:9851-9855; R.G. Vogt, R. Rybczynski and M.R. Lerner [1991] J. Neurosci. 11:2972-2984; M.P. McKenna, Hekmal-Scafe, P. Gaines and J.R. Carlson [1994] J. Biol. Chemistry 269:16340-16347; and C.W. Pikielny, G. Hasan, F. Rouyen, and M. Rosbash [1994] Neuron 12:35-49). Arrows above yellow highlighting denotes conserved cysteine residues found in all 12 aligned sequences. Yellow highlighting with no arrow denotes cysteine residues conserved in the insect pheromone binding proteins, but not in the B proteins, 2-2, or Tm 13.17. Red shading shows agreement between one or more of the Tm 13.17, 2-2, or B1/B2 sequences and any of the representative pheromone or odorant binding proteins. (Pbp: pheromone binding protein; Obp: oderant binding proteins, Antpo (Antherea polyphemus); Manse (M. sexta), Drome, Drosophila melanogaster).--

Please amend the paragraph beginning on page 26, line 27 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 4.18 illustrates the areas of repeated similarity surrounding the conserved cysteine residues of 2-2, 2-3, 3-4, 3-9, 7-5, Tm 13.17 (SEQ ID NO:1; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:13, SEQ ID NO:14; SEQ ID NO:3; SEQ ID NO:4), B1, B2, (Paesen G. C., and G. M. Happ [1995] Insect Biochem. Molec. Biol. 25: 401-408) and AFP-3 (Tang and Baust, [1995] GenBank NCBI SEQ ID NO:785071). Conserved cysteine residues are in yellow. Lysine residues are

shown in red, glutamate in green, isoleucine in orange, and valine in blue.--

Please amend the paragraph beginning on page 28, line 21 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 5.7 describes the specific cDNA nucleotide sequence ~~(SEQ. ID NO. 16)~~ (SEQ ID NO:16) and translation precursor protein ~~(SEQ ID NO. 17)~~ (SEQ ID NO:17) of His-tagged signal plus 2-2 clone. The signal sequence is underlined, and bold "1" denotes the predicted beginning of the mature protein. The start codon is labeled, and the stop codon is denoted with a star.--

Please amend the paragraph beginning on page 28, line 25 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 5.8 describes the specific cDNA nucleotide sequence ~~(SEQ. ID NO. 18)~~ (SEQ ID NO:18) and translation of mature peptide ~~(SEQ ID NO. 19)~~ (SEQ ID NO:19) of His-tagged signal minus 2-2 clone. The His-tag is upstream of the N-terminal of the mature protein. The bold "1" denotes the predicted beginning of the mature protein. The stop codon is denoted with a star.--

Please amend the paragraph beginning on page 28, line 29 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 5.9 describes the specific cDNA nucleotide sequence ~~(SEQ. ID NO. 20)~~ (SEQ ID NO:20) and translation precursor protein ~~(SEQ ID NO. 21)~~ (SEQ ID NO:21) of His-tagged signal plus 2-3 clone. The signal sequence is underlined, and bold "1" denotes the predicted beginning of the mature protein. The start codon is labeled, and the stop codon is

denoted with a star.--

Please amend the paragraph beginning on page 28, line 33 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 5.10 describes the specific cDNA nucleotide sequence ~~(SEQ. ID NO. 22)~~ (SEQ ID NO:22) and translation of mature peptide ~~(SEQ ID NO. 23)~~ (SEQ ID NO:23) of His-tagged signal minus 2-3 clone. The His-tag is upstream of the N-terminal of the mature protein. The bold "1" denotes the predicted beginning of the mature protein. The stop codon is denoted with a star. --

Please amend the paragraph beginning on page 28, line 37 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 5.11 describes the specific cDNA nucleotide sequence ~~(SEQ. ID NO. 24)~~ (SEQ ID NO:24) and translation precursor protein ~~(SEQ ID NO. 25)~~ (SEQ ID NO:25) of His-tagged signal plus Tm 13.17 clone. The signal sequence is underlined, and bold "1" denotes the predicted beginning of the mature protein. The start codon is labeled, and the stop codon is denoted with a star.--

Please amend the paragraph beginning on page 29, line 3 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 5.12 describes the specific cDNA nucleotide sequence ~~(SEQ. ID NO. 26)~~ (SEQ ID NO:26) and translation of mature peptide ~~(SEQ ID NO. 27)~~ (SEQ ID NO:27) of His-tagged signal minus Tm 13.17 clone. The His-tag is upstream of the N-terminal of the mature protein. The bold "1 " denotes the predicted beginning of the mature protein. The stop codon is denoted with a star.--

Please amend the paragraph beginning on page 29, line 19 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

-- ~~FIG. 7-1~~ FIG. 7.1 is a table listing of letter designations for amino acids and chemical classifications.

Please amend the paragraph beginning on page 29, line 21 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--~~FIG. 7-2~~ FIG. 7.2 describes specific details of the nucleotide cons[[c]]ensus sequences developed for the Tm 12.86 family of genes (SEQ ID NO:44; SEQ ID NO:45; SEQ ID NO:46; SEQ ID NO: 47).--

Please amend the paragraph beginning on page 29, line 23 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--~~FIG. 7-3~~ FIG. 7.3 describes specific details of the protein cons[[c]]ensus sequences encoded by the Tm 12.86 family of genes (SEQ ID NO:48).–

Please amend the paragraph beginning on page 29, line 25 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--~~FIG 8.0~~ FIG. 8.0A illustrates the recrystallization of H_2O after 1 minute; FIG. 8.0B after 30 minutes; and FIG. 8.0C after 2 hours. FIG. 8.0D illustrates the recrystallization of NaCl after 1 minute; FIG. 8.0E after 30 minutes; and FIG. 8.0F after 2 hours. ~~(left) and NaCl (right) occurring after 1 minute, 30 minutes, and 2 hours respectively~~. All samples were annealed at -60.degree. C. (bars=0.1 mm).--

Please amend the paragraph beginning on page 29, line 28 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--~~FIG. 8.1 is a low magnification view of a splat-cooled 0.9% NaCl sample annealed at -6.degree. C. for 30 minutes.~~ FIG. 8.1a[[A.]] is a low magnification view of a splat-cooled 0.9% NaCl sample annealed at -6.degree. C. for 30 minutes. Center (c), mid-sample (m), and edge (e) regions are shown. The sample is resting on a support ring (arrow). "th"=thermocouple. (bar at lower right=3.0 mm). FIG. 8.1b[[B.]] is a low magnification view of a splat-cooled 0.9% NaCl sample annealed at -2.degree. C. for 30 minutes. Putative maximum deformation (mxd) and minimum deformation (mnd) areas are shown. (bar =1.0 mm).--

Please amend the paragraph beginning on page 32, line 26 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 8.37A is a comparision of time course of recrystallization plots for experimental ~~and theoretical~~ prediction using log/log transformations. FIG. 8.37B is a comparision of time course of recrystallization plots for theoretical prediction using log/log transformations. --

Please amend the paragraph beginning on page 33, line 8 as indicated. Note: Figure designations were underlined in the original application, and remain unchanged in this amendment.

--FIG. 8.43 illustrate regions of Tm 13.17 clone used as DNA probes (SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4). Color coded areas denote forward and reverse ~~prmer~~ primer sequences used for particular experiments with the regions between and including primer sequences denoting the probe. Probe outline by yellow region was used in Example 4, probe from green region used in Example 5, and probe from pink region used for northern analysis.--

Please amend the paragraph beginning on page 33, line 13 as indicated. Note: Figure designations were underlined in the original application, and in this amendment, both Figure designations are now underlined.

--FIG. 8.44 illustrate regions of Tm 2-2 clone used as DNA probes (SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:8). Color coded areas denote forward and reverse ~~prmer~~ primer sequences used for particular experiments with the regions between and including primer sequences denoting the probe. Probe usage as in FIG. 8.43.--

Please amend the paragraph beginning on page 36, line 24 as indicated.

--Amino-terminal sequence analysis for Tm 12.86 revealed the sequence for the first nineteen amino acids from the amino terminus ~~SEQ ID NO 1~~ SEQ ID NO:1 and indicated leucine as the amino-terminal amino acid (FIG. 1.8). This result provided added confirmation that Tm 12.86 is a single protein species. To investigate the possibility that a carbohydrate component was associated with Tm 12.86, an additional SDS-PAGE was conducted and stained with PAS.--

Please amend the paragraph beginning on page 40, line 38 as indicated.

--The isolation and characterization of Tm 12.86, and the obtainment of a highly specific and sensitive antibody generated against it, were necessary prerequisites for implementing molecular studies to isolate the gene encoding for this AFP. Steps were taken to construct cDNA libraries from mRNA populations containing the message for Tm 12.86, from whole animal and fat body derived from cold acclimated *T. molitor* larvae according to the procedures detailed in Example 2. Immuno-screening with Tm 12.86 antibody identified a cDNA clone that was subsequently isolated and characterized ~~(SEQ ID NO. 2)~~ (SEQ ID NO:2) and found to encode for a distinct protein, Tm 13.17 ~~(SEQ ID NO. 3~~ (SEQ ID NO:3 (precursor peptide) ~~SEQ ID NO. 4~~ SEQ ID NO:4 (mature peptide)). The N-terminal sequence of Tm 13.17 shows 61% identity, 83%

similarity with that of Tm 12.86 ~~(SEQ ID NO. 1)~~ (SEQ ID NO:1), indicating that this clone is a homologous gene to that of Tm 12.86.--

Please amend the paragraph beginning on page 45, line 38 as indicated.

--DNA sequence analysis and similarity search. All of the seven clones were initially partially sequenced manually from both strands. All were found to have identical DNA sequence. The FW1 clone was then selected for a complete DNA sequence determination of both strands by automatic sequencing. The nucleotide sequence ~~(SEQ. ID No 2)~~ (SEQ ID NO:2) and deduced amino acid sequence ~~(SEQ. ID Nos 3 and 4)~~ (SEQ ID NO:3 and SEQ ID NO:4) of FW1 is presented in FIG. 2.6a. The full length of the cDNA of the FW1 clone is 577 nucleotides long and contains the cloning site E coR I at position 13 and XhoI at position 530. From the partial sequences of the 6 other clones, no sequence heterogeneity was found from that of the clone of FW1, indicating they all contain the same insert cDNA of *T. molitor*. There is one open reading frame (ORF) from the 577 base pairs. Its start codon ATG is 35 nucleotides downstream from the 5'-end of the clone and the stop codon TGA is at the position of 438 base pair. The 402 nucleotides within encode a peptide containing 134 amino acid residues with a molecular weight of 15.128 kDa. This also includes a putative signal peptide at the N-terminus with 18 amino acid residues, which shows characteristics typical of other signal peptide sequences, including three distinct regions: a basic positively charged N-terminal region (n-region); a central hydrophobic region (h-region) and a more polar C-terminal region (c-region) (FIG. 2.6b). Thus, the predicted mature protein is of 116 amino acid residues ~~(SEQ ID No. 4),~~ (SEQ ID NO:4), with a molecular weight of 13.17 kDa derived from 348 nucleotides. The mature peptide is designated as Tm13.17 for *T. molitor* 13.17 kDa molecular weight. The 3'-end untranslated region of 139 nucleotides is A-T rich (A:T:C:G=55:31:27:26) and presents a AATAAA polyadenylation signal which is located 49 nucleotides downstream of the stop codon and 13 nucleotides upstream of the poly (A) tail. The poly (A) tail occurs 26 nucleotides downstream of the polyadenylation signal.--

Please amend the paragraph beginning on page 47, line 4 as indicated.

--Similarity of the NH2 terminus between Tm 13.17 and Tm 12.86. A comparison of the N-terminal sequence of Tm 13.17 with that determined from protein analysis of Tm 12.86 ~~(SEQ ID NO. 1)~~ (SEQ ID NO:1) indicates a very strong degree of relatedness (FIG. 2.10). 11 out of 18 N-terminal amino acid residues are identical between Tm 13.17 and Tm 12.86. Moreover, in addition to the identical amino acid residues there are 4 highly conservative replacements. Thus, the N-terminus of these two AFPs shows an identity of 61% and similarity of 83%.--

Please amend the paragraph beginning on page 52, line 13 as indicated.

--Nucleotide sequencing for clones 2-2 ~~(SEQ. ID NO. 5)~~ (SEQ ID NO:5) and clone 2-3 ~~(SEQ. ID NO. 6)~~ (SEQ ID NO:6) and predicted amino-acid residues ~~(SEQ. ID NO. 7 and 8)~~ (SEQ ID NO:7 and SEQ ID NO:8) for clones 2-2 and 2-3 are shown in FIG. 3.0 for clone 2-2 and FIG. 3.1 for clone 2-3. The 2-2 cDNA insert consists of a sequence 482 bp. in length, while the 2-3 full cDNA sequence is 483 bp. in length. An evaluation of amino acid translation of the 2-2 cDNA sequence using all six possible reading frames revealed only one likely open reading frame (ORF) consisting of 133 amino acids. An identical amino acid sequence was deduced for 2-3. Toward the start of the ORF for 2-2 and 2-3, a sequence of 18 amino acids corresponds exactly with the amino terminus sequences of Tm 12.86 ~~(SEQ. ID NO. 1,~~ (SEQ ID NO:1, FIG. 1.8). Preceding this 18 amino acid sequence within 2-2 and 2-3 is another 18 amino acids (FIGS. 3.0 and 3.1) that constitute a putative signal peptide characteristic of proteins synthesized for export.--

Please amend the paragraph beginning on page 61, line 2 as indicated.

--A total of five new immunopositive clones were sequenced. Many more positive clones were observed (on average seven per plate in the primary screening), but due to the

inherent difficulty in separating the positives from the background plaques, and the need for secondary and tertiary screenings, only five were eventually isolated. Out of the five, two of these clones appear to be false positives, since their sequences are unrelated to Tm 13.17 or 2-2 and 2-3. These may be due to endogenous peroxidases that were not completely knocked out by the peroxide treatment. The remaining three clones were nearly identical in nucleotide sequence to the existing 2-2 and 2-3 clones, and were designated 3-4, 3-9, and 7-5 (FIG. 4.10, 4.11, and 4.12) having ~~SEQ ID NO's 9, 12, and 15~~ SEQ ID NOS:9, 12, and 15 respectively, and encoding for peptides (precursor and mature) having ~~SEQ ID NO's 10-11, 13-14, and 7-8,~~ SEQ ID NOS:10-11, SEQ ID NOS:13-14, and SEQ ID NOS:7-8, respectively for each clone.--

Please amend the paragraph beginning on page 68, line 12 as indicated.

--Generation of Signal Peptide Deleted Fragment(s). Signal peptide deleted fragments were generated by PCR with primers designed to sequences downstream of the signal peptide and upstream of the stop codon. Additionally, two artificial restriction sites, BamHI and XhoI, were designed in the primers in order to incorporate these sites in the fragments ~~(SEQ ID NO's 40-43).~~ (SEQ ID NOS:40-43). The plasmid DNA isolated in the previous step was used as a template in the PCR reaction. Following PCR, the entire reaction product was then electrophoresed on a 1.5% agarose gel, and a distinct and strong band was observed at 350 bp. Since this is the expected size of the AFP clones when the signal peptide, poly-A tail and other non-coding regions are removed, this result suggests that the primers and the PCR reaction successfully yielded a signal deleted cDNA fragment.--

Please amend the paragraph beginning on page 70, line 26 as indicated.

--Sequencing of PET-AFP vectors. For final confirmation that signal-plus and signal-deleted inserts were successfully subcloned into the pET vector without accruing mutations, the

sequence analyses of plasmids were performed. Plasmids from bacterial stocks of pET-AFP clones were extracted using procedures detailed in Example 5. The plasmids were amplified by using the T7 promoter sequence found in the upstream region of the multiple cloning site. Following this, sequence analysis of the clones was conducted on a ABI Prism Sequencer. The positive control was pET vector without any insert. The results were compared with the original sequences and were found to have no error. Some sequences were unrecognized by the software and manually read and verified for accuracy. In addition, the sequences encoding for the histidine tag, the thrombin cleavage site and the 17 tag were preserved in all the clones. The sequencing results of pET-[2-2S+, 2-2S-, 2-3S+, 2-3S-, Tm 13.17S+ and Tm 13.17S-] are presented in FIGS. 5.7-5.12 ~~(SEQ ID NO's 16-27)~~ (SEQ ID NOS:16-27) FOR NUCLEOTIDE AND PEPTIDE SEQS.--

Please amend the paragraph beginning on page 77, line 35 as indicated.

--Cons[[c]]ensus sequences for the genes and proteins of the Tm 12.86 family (cladistic tree shown in FIG. 4.20) were identified as detailed in Example 7 paying careful attention to the types of substitutions and chemistry involved. Both a full general cons[[c]]ensus sequence was described for the entire Tm 12.86 gene family encoded proteins, and consensus sequences for the nested genes within the family are also described (i.e. cons[[c]]ensus sequence for Tm 12.84-6 like, consensus sequence expanded to include Tm 13.17 like, cons[[c]]ensus sequence expanded to include B1/B2 like, and cons[[c]]ensus sequence expanded to include AFP-3 like, genes and their encoded proteins ~~(SEQ ID NO's 44-48).~~ (SEQ ID NOS:44-48). Detailed in FIGS. 7.2 and 7.3 are the full breath of the cons[[c]]ensus sequences for nucleotides and amino acids, respectively, and for each grouping the most representative cons[[c]]ensus sequence, and also positions and types of substitutions either occurring or deemed acceptable. See FIG. 7.1 for reference to amino acid letter designations and chemical classifications.--

Please amend the paragraph beginning on page 78, line 9 as indicated.

--The 5 clones in this series are highly conserved. At the protein level, one (3-9) shows a substitution at position 37 (from the initial methionine) of an amino acid with an acidic side chain (glutamic acid) for one with an aliphatic side chain (valine). Since valine is the most common, it is placed in the cons[[c]]ensus sequence, with the understanding that glutamic acid is a recognized substitution for this gene family. Clone 3-9 also shows a substitution at position 69 of an amino acid with a basic side chain (arginine) for another with a basic side chain (lysine). Again, since lysine is most common, it is included in the cons[[c]]ensus, with arginine a recognized and expected substitution. Another clone (3-4) shows a substitution at position 122** of an amino acid with a hydrophobic sulf[[p]]hydryl group (cysteine) with another having a hydrophobic, aliphatic side chain (valine). Since cysteine is most common it is included in the cons[[c]]ensus with valine noted as a potential substitution. For alignment purposes in FIG. 7.3, a gap is present at position 94 in the sequence for ALL Tm 12.84 clones, since they share the smaller, 115 residue number. Thus, as will be the case for all Tm 12.84 clones, residue position numbers in FIG. 7.3, listed after 94 will reflect this extra number assignment. Therefore, as in the example above, clone 3-4 has the valine substitution actually at position 121 from the initial methionine, as seen in ~~SEQ ID NO. 10)~~ SEQ ID NO:10).--

Please amend the paragraph beginning on page 79, line 18 as indicated.

--Together, the conserved residues and similar substitutions form a general pattern that contributes to the special chemistry of this family of proteins, including their ability to bind to ice and prevent crystal growth. ~~SEQ ID NO. 48~~ SEQ ID NO:48 presents a full general consensus peptide sequence for the entire Tm 12.86 gene family. With this in mind, although never tested, the close similarity of the B1 and B2 *T. molitor* proteins (indeed more so than AFP-3) suggest that these will likely exhibit antifreeze activity.--

Please amend the sentence beginning on page 102, line 34 as indicated.

--For the selected *T. molitor* hemolymph samples represented in FIGS. 8.23, 8.25 and 8.30, the increase in hemolymph RI factor associated with the acclimation of *T. molitor* from summer to winter conditions is observed as leftward shifts of the regression lines.--

Please replace the paragraph beginning on page 145, line 8 with the following amended paragraph. Note: The words Sequence Data and T. molitor were underlined in the original specification. Added material starts with the word Genbank's.

-- Sequence Data. DNA sequence data from *T. molitor* was obtained from cDNA clones selected from a *T. molitor* cold acclimated cDNA library with an antibody to the T. molitor AFP Tm 12.86. Several positive clones were sequenced using the ABI Prism model 310 DNA sequencer. The clones concentrated on are Tm 13.17 (Example 2), 2-2 and 2-3 (Example 3), and 3-4, 3-9, and 7-5 (Example 4, Part C). Also available were the N-terminal amino acid sequence of Tm 12.86 (Example 1), and the nucleotide sequence and predicted amino acid sequence of AFP-3, B1 and B2, and other sequence data obtained from GenBank's database located on the National Center for Biotechnology Information's website - www.ncbi.nlm.nih.gov [~~GenBank (www.ncbi.nlm.nih.gov)~~]. --

Please amend the paragraph beginning on page 158, line 12 as indicated.

--In developing cons[[c]]ensus sequences for the genes and proteins of the Tm 12.86 family (cladistic tree shown in FIG. 4.20), careful attention was made to the types of substitutions and the chemistry involved. Both a full generic cons[[c]]ensus sequence was identified for the entire Tm 12.86 gene family encoded proteins, and consensus sequences for the nested genes within the family are also described (i.e. cons[[c]]ensus sequence for Tm 12.84-6 like, consensus sequence expanded to include Tm 13.17 like, cons[[c]]ensus sequence expanded to include B1/B2 like, and cons[[c]]ensus

expanded to include AFP3 like, genes and their encoded proteins ~~(SEQ ID NO. 44-48).~~ (SEQ ID NOS:44-48).--

Please amend the paragraph beginning on page 165, line 12 as indicated.

--The written Sequence Listings for ~~SEQ ID NO's 1-48~~ SEQ ID NOS:1-48 (pages 166-221) are attached herein with the Submission of the Computer Readable Copy.--